Influence of Purinoceptor Antagonism on Diadenosine Pentaphosphate-Induced Hypotension in Anesthetized Rats\textsuperscript{1}

MARTIN STEINMETZ, TRUC VAN LE, PETER HOLLAH, GERT GABRIELS, HELGE HOHAGE, KARL HEINZ RAHN, and EBERHARD SCHLATTER

Medizinische Poliklinik, Experimentelle Nephrologie, Westfälische Wilhelms-Universität, Münster, Germany

Accepted for publication May 26, 2000 This paper is available online at http://www.jpet.org

ABSTRACT

Diadenosine polyphosphates (ApnA; \( n = 3–6 \)) are potent vasoactive agents in isolated vessels. Information on effects of ApnA in vivo is still limited despite the fact that these compounds are starting to be used in humans. This study was designed to compare the effects of ApnA and their possible metabolites on blood pressure in vivo and to functionally identify purinoceptors involved in their action. All four ApnA and their degradation products induced a sustained drop of mean arterial blood pressure during i.v. infusion, which was fully reversible. The rank order of potency was Ap4A \( \geq \) Ap6A \( > \) Ap5A = Ap3A = ATP = ADP \( > \) AMP \( \geq \) adenosine, suggesting that the hypotensive effect is predominantly evoked by the original dinucleotides and not by their degradation products. The hypotensive effect of Ap5A was reduced by the P2X and P2Y\textsubscript{1} purinoceptor antagonist pyridoxal-phosphate-6-azophenyl-2'-4'-disulfonic acid, the A\textsubscript{1} purinoceptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, and the A\textsubscript{2} purinoceptor antagonist 3,7-dimethyl-1-propargylxanthine. The hypertensive effect by the prototype P2X receptor agonist \( \alpha\beta\)-methylene ATP was inhibited by pyridoxal-phosphate-6-azophenyl-2'-4'-disulfonic acid, too. Purinoceptor antagonists reduced the maximal effects of the agonists indicating a noncompetitive inhibition. In summary, the reported vasocontractile effect of ApnA seems to be limited to isolated preparations under resting tone conditions; however, the systemic cardiovascular effects of all four ApnA are hypotensive, also making them candidates for blood pressure reduction in humans. These effects are fast in onset and easily reversible. Activation of different purinoceptors in the vasculature (most probably P2Y\textsubscript{1} and A\textsubscript{2} receptors) contributes to the Ap5A-induced decrease of mean arterial blood pressure.

Diadenosine polyphosphates (ApnA) act as humoral signal transducers and neurotransmitters and are coreleased with ATP and catecholamines (Castillo et al., 1992; Sillero et al., 1994). ApnA are stored in and released from human platelets (Schütler et al., 1994) and degradation half-time in the blood is considerably longer than that for ATP, the prototype purinergic agent (Lüthje and Ogilvie, 1988). The naturally occurring forms have a chain length of two to seven phosphate groups. These vasoactive purines exert various effects in isolated kidney and heart preparations, in isolated resistance vessels, or in endothelial cells (Busse et al., 1988; Vahlensieck et al., 1996, 1999; van der Giet et al., 1997; Stachon et al., 1998; Steinmetz et al., 2000a,b). These effects include vasoconstriction as well as vasodilation, suggesting a role in blood pressure regulation. Vasodilation seems to be more pronounced in raised tone preparations, whereas vasoconstriction is seen mostly under resting tone conditions. Most studies so far have examined the vasoactive effects of ApnA in isolated vessel or organ preparations and little information is available on systemic actions of these compounds in vivo. In anesthetized rats we showed that i.v. bolus injection of Ap4A, Ap5A, or Ap6A induced a transient decrease of heart rate and cardiac output. This is accompanied by a short-term rise of total peripheral resistance followed by a long-lasting significant reduction of total peripheral resistance and a simultaneous marked fall in blood pressure (Khattab et al., 1998). Recently the first use of ApnA in anesthetized humans was described were Ap4A caused a sustained drop in blood pressure (Kikuta et al., 1999). This illustrates the growing pharmacological relevance of naturally occurring purinergic agents such as ApnA and the urgent need of a better understanding of their pharmacological effects in vivo.

ABBREVIATIONS: ApnA, diadenosine polyphosphates; PPADS, pyridoxal-phosphate-6-azophenyl-2'-4'-disulfonic acid; DMPX, 3,7-dimethyl-1-propargylxanthine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MABP, mean arterial blood pressure; HR, heart rate; BPM, beats per minute; Ap3A, P\textsuperscript{1},P\textsuperscript{3}-diadenosine triphosphate; Ap4A, P\textsuperscript{1},P\textsuperscript{4}-diadenosine tetraphosphate; Ap5A, P\textsuperscript{1},P\textsuperscript{5}-diadenosine pentaphosphate; Ap6A, P\textsuperscript{1},P\textsuperscript{6}-diadenosine hexaphosphate.
In isolated organ and vessel preparations smooth muscular P2X receptors were found to be responsible for purinergic vasoconstriction, whereas purinergic vasodilation was due to P2Y and A2 purinoceptors on the smooth muscle cells or the endothelium (Busse et al., 1988; Abbracchio and Burnstock, 1994). To better understand the effects and pharmacodynamics of these purinergic agents when given systemically we investigated the influence of ApnA (n = 3–6) and of their possible degradation products (ATP, ADP, AMP, and adenosine; Lüthje and Ogilvie, 1988) as well as of the prototype P2X purinoceptor agonist αβ-methylene ATP (Fredholm et al., 1994; Humphrey et al., 1998), but also P2Y1 receptor antagonists (Vigne et al., 1998), DMPX (3,7-dimethyl-1-propargylxanthine) as A2 receptor antagonist (Fredholm et al., 1994), and DPCPX (8-cyclopentyl-1,3-dipropylxanthine) as A1 receptor antagonist.

Materials and Methods

Experimental Design. Male Wistar rats (250–400 g) obtained from Charles River, Sulzfeld, Germany, were used. The animals had free access to water and standard diet. There were prepared for measurements of mean arterial blood pressure (MABP) and heart rate (HR). In a first series these parameters were measured after different modes of drug application (i.v. bolus injection, i.a. bolus injection, or i.a. infusion). These data were compared with those of a recent study (Khattab et al., 1998) in which we investigated the cardiovascular effects of ApnA after i.v. bolus injection.

To compare the effects of Ap5A, Ap4A, Ap5A, and Ap6A and the possible degradation products of ApnA (ATP, ADP, AMP, and adenosine) on MABP and HR, these substances were administered to a second group of animals via i.v. infusion.

In a third series the involvement of purinoceptors was studied. Because Ap5A is the most potent vasoconstrictive ApnA at basal tone and the most potent vasodilative after phenylephrine precontraction in isolated vessel preparations (Steinmetz et al., 2000b), blood pressure changes induced by i.v. infusion of Ap5A and of ATP, ADP, AMP, adenosine, or αβ-methylene ATP were antagonized by PPADS, DMPX, or DPCPX. Antagonists were infused i.v. 5 min before and during application of the agonists, with only one type of antagonist infused in the same animal. Each dose tested was examined in three to six rats, which were different from those of series 2. Each animal received up to five different doses of the substances given in randomized order with control periods of at least 10 min between two periods of drug infusion. Each agonist was infused for 5 min; in a few additional experiments up to 10 min. Effects were always compared with averaged basal values before and after drug application.

Surgical Procedures. All animals were anesthetized with urethane (1.6–2 g/kg i.p.) and operated on a thermostatically controlled animal operation table (TSE GmbH, Bad Homburg, Germany) at 37°C. A polyethylene catheter was inserted into the right carotid artery for MABP measurements by use of a pressure transducer connected to a computer-aided small animal pressure gauge (Transonic Systems Inc., Ithaca, NY). Another polyethylene catheter inserted into the jugular vein was used for isotonic saline infusion or drug application. For drug infusion a perfusion pump (60 μl/min, Perfusor; Braun, Melsungen, Germany) was used. All drugs were dissolved in isotonic saline. HR was measured with a computer-aided biological monitoring system (BMON; TSE GmbH). An equilibrium time of 45 to 60 min was allowed after surgical procedure before drug application.

Determination of Blood Levels of Ap5A. Blood samples were collected by venipuncture of the inferior caval vein after medial opening of the abdominal wall, with 10-ml monovettes containing EDTA (1.2–2 mg/ml blood; Saarstedt, Nümbrecht, Germany), being placed in ice water immediately after collecting the sample. Sample preparation and measurement of Ap5A levels by HPLC were performed as reported before in detail (Schlüter et al., 1994; Hollah et al., 1998).

Statistics. Data are presented as mean ± S.E. Significances were tested with multiple parametric ANOVA test, with P < .05 denoting statistical significance.

Results

General Experimental Data and Comparison of Application Modes. The MABP of the animals at control conditions was 115 ± 8 mm Hg. MABP was stable for at least 2.5 h before a slow, continuous decrease was observed. Application of Ap4A as i.v. infusion resulted in a dose-dependent and reversible decrease of MABP that was not significantly different from the maximal decreases of MABP induced by i.a. infusion or i.a. bolus injection (data not shown). The effects also were not significantly different from those seen after i.v. bolus injection, which we reported before (Khattab et al., 1998). The only difference was that after bolus injection MABP decreased transiently and recovered within 2 min, whereas in the infusion experiments, it lasted as long as the drug was infused. Figure 1 shows original recordings of MABP illustrating the immediate blood pressure-lowering effect of Ap5A (27, 109, and 545 nmol/kg·min) during continuous i.v. infusion. The hypotensive effect was maintained during the whole period of application and the initial MABP was restored within 1 to 2 min after omission of the drug from the infusion with a slight transient increase above the initial value. The concentration of Ap5A in the blood drawn from the animals by venipuncture of the inferior
caval vein immediately after 5 min of i.v. infusion (545 nmol/kg·min) was 1.34 ± 0.20 μmol/l.

**Effects of i.v. Infusion of Purinergic Agonists on MABP.** All agonists induced a significant and sustained drop of MABP. The rank order of potency was Ap4A > Ap6A > Ap5A = Ap3A = ATP = ADP > AMP > adenosine (Fig. 2). The hypotensive effects of the possible degradation products of the ApnA were weaker than that of Ap4A and of Ap6A.

The hypotensive effects reached with the maximal doses applied ranged between −29.4 ± 3.5 mm Hg (Ap4A) and −5.9 ± 0.4 mm Hg (adenosine). There was no significant difference between the half-time constants of the different substances to decrease MABP. Likewise, half-time constants for the return to the baseline values after stopping the infusion were not significantly different between all ApnA and their potential metabolites. HR that was 367 ± 9 (n = 19) beats per min (BPM) under resting conditions decreased significantly on infusion of ApnA. At the highest doses used Ap6A reduced HR by 51 ± 13 BPM (Ap5A, 48 ± 10 BPM; Ap4A, 46 ± 15 BPM; Ap3A, 50 ± 12 BPM). HR returned to baseline levels within 1 min (data not shown) despite the continuous presence of the agonist and was not analyzed further in this study.

**Inhibitory Effects of P2 Purinoceptor Antagonist PPADS.** Figure 3 is an original trace showing the inhibitory effect of PPADS (70 nmol/kg·min) on the hypotension induced by Ap5A (55 nmol/kg·min), reducing the drop of MABP by approximately 50%. Low concentrations of PPADS (up to 70 nmol/kg·min) given alone did not significantly alter MABP (70 nmol/kg·min, 2 ± 1 mm Hg), whereas high concentrations of PPADS (690 nmol/kg·min) increased blood pressure by 9 ± 3 mm Hg. In Fig. 4, the dose dependence of the inhibitory effect of PPADS on Ap5A-induced hypotension is depicted. The maximum of the drop in MABP by Ap5A is reduced by PPADS, which suggests a noncompetitive inhibitory mechanism of PPADS. PPADS (138 nmol/kg·min) also decreased the adenosine-induced (187 nmol/kg·min) drop of MABP by 44 ± 8% and the ATP-induced (182 nmol/kg·min) drop of MABP by 34 ± 13%, but did not influence the hypotensive effects of AMP or ADP (data not shown).

In contrast to Ap5A the P2X purinoceptor agonist αβ-methylene ATP increased MABP by 44 ± 5 mm Hg at a dose of 99 nmol/kg·min. PPADS also inhibited this hypertensive effect of αβ-methylene ATP. It did so, however, at much higher doses (345–690 nmol/kg·min) than required for inhibition of the hypotensive effect of Ap5A. The dose-response curves again indicate a noncompetitive mechanism of inhibition (Fig. 5).

**Inhibitory Effects of P1 Purinoceptor Antagonists.** DPCPX and DMPX alone did not exert any significant influence on MABP. The A1 receptor antagonist DPCPX had the highest potency to inhibit the Ap5A-induced decrease of MABP because a dose of 33 nmol/kg·min induced the inhibition of blood pressure drop by 65 ± 7% (Fig. 6), whereas the A2 purinoceptor antagonist DMPX achieved a similar inhibitory effect only at a dose of 458 nmol/kg·min (Fig. 7); for comparison, PPADS had an equipotent inhibitory effect at a dose of 70 nmol/kg·min (Fig. 4).

DPCPX (33 nmol/kg·min) also decreased the drop of MABP induced by adenosine (187 nmol/kg·min) by 23 ± 7%, that induced by ATP (182 nmol/kg·min) by 47 ± 16%, and that induced by ADP (222 nmol/kg·min) by 30 ± 17% but did not significantly influence the hypotensive effect of AMP (data not shown).
DMPX (458 nmol/kg · min) decreased the drop of MABP induced by adenosine or ATP (182 nmol/kg · min each) by 32 ± 6% or 66 ± 6%, respectively. The effects of AMP (143 nmol/kg · min) or ADP (222 nmol/kg · min) were not influenced (data not shown).

**Discussion**

In a former study Ap4A was the most potent ApnA (n = 4–6) to reduce MABP in anesthetized rats after i.v. bolus injection (Khattab et al., 1998). Herein, we could demonstrate that there is no significant difference between the maximal reductions of MABP after i.v. infusion of Ap4A compared with i.a. infusion or bolus injection or i.v. bolus injection (Khattab et al., 1998). This indicates that there are most likely no significant secondary effects due to either metabolism or compartmentalization of Ap4A. Furthermore, it appears that no difference whether Ap4A reaches the heart first before entering arterial resistance vessels or vice versa. In all further experiments presented in this report the purinergic agents were infused i.v. because this mode of application allows to establish a steady-state condition (Fig. 1) and also a well-balanced systemic distribution of the substances into all vascular beds of the organism. This is of importance because the vasoactive effects of ApnA depend on the type of vascular bed under examination (Steinmetz et al., 2000b). All agonists examined (with the exception of αβ-methylene ATP) reduced MABP. The rank order of potency was Ap4A > Ap6A > Ap5A = ATP = ADP > AMP > adenosine. With the onset of MABP decrease a short transient reduction of HR was regularly observed. However, throughout the following hypotensive period the initial HR was restored as described in Khattab et al. (1998). ApnA are asymmetrically degraded to ATP, ADP, or AMP, and finally to adenosine. Therefore, one has to consider that the observed effects of ApnA could be mediated by these metabolites. However, the much higher potency and efficacy of Ap6A and Ap4A compared with their monoadenosine nucleotide degradation products or adenosine indicate that the effects are due to the original agents. Furthermore, the degradation half-time of ApnA in blood is significantly longer than that of ATP or ADP and exceeds the time of infusion of ApnA in this study (Lütjhe, 1989). After bolus injection of Ap4A, Ap5A, and Ap6A the blood levels were below detection limits (10 pM; Khattab et al., 1998), whereas after i.v. infusion in this study Ap5A concentrations in the blood reached the micromolar range, which is high enough to mediate the observed effects. Furthermore, there were no significant differences in the time course of the onset and the velocity of the blood pressure decrease or in the recovery between all tested agonists. This would have been observed if ApnA act mostly via their degradation products. Taken together, these arguments strongly suggest that at least a large portion of the blood pressure-lowering effects is mediated by the ApnA themselves, although some influence of their possible degradation products cannot be excluded. This conclusion is in line with several other studies on effects of ApnA in vivo or in isolated organ preparations (Busse et al., 1988; Lütjhe and Ogilvie, 1988; Pohl et al., 1991; Schlüter et al., 1994; Vahlensieck et al., 1996, 1999; van der Giet et al., 1997). Remarkably, the systemic effects of ApnA in anesthetized rats are purely hypotensive. Vasoconstrictive effects of ApnA do occur, however, on the local vascular level according to a number of studies describing the vascular effects of ApnA in isolated arterial vascular beds (Pohl et al., 1991; Ralevic et al., 1995; van der Giet et al., 1997; Steinmetz et al., 2000b). The vasoconstrictive effects of ApnA in these isolated vessels were, however, most prominent under resting tone conditions. In some of these studies Ap5A proved to be the most potent vasoconstrictor of all ApnA. Short phosphate chain ApnA (Ap3A and Ap4A) caused vasodilations only in some studies. In precontracted, isolated rat resistance arteries all ApnA induced a small transient vasoconstriction followed by a marked vasodilation (Steinmetz et al., 2000a,b). Ap5A was
found to be the most potent vasoconstrictor as well as the most potent vasodilator. Therefore, and because of comparatively high concentrations found releasable from thrombocytes (Jankowski et al., 1999), Ap5A was chosen herein as exemplary of ApnA to be antagonized by the purinoreceptor antagonists.

PPADS initially was looked at as a selective P2X receptor antagonist (Windscheiff et al., 1994). Obviously, this P2X receptor antagonism is the reason for the inhibition of the hypertensive effect of αβ-methylene ATP observed in this study. The dose-response curves suggest a noncompetitive antagonism by PPADS as reported by Czeche et al. (1998). The PPADS-triggered antagonism of the hypertensive effect of Ap5A at concentrations 10 times lower than those necessary for the inhibition of αβ-methylene ATP is probably not due to P2X receptor antagonism because purinergic relaxation of vascular smooth muscle is known to be mainly P2Y receptor-dependent. In addition, P2Y2 receptor antagonist competitive qualities of PPADS have been reported (Vigne et al., 1998). Taken together, this suggests a nonselective P2 receptor antagonism by PPADS in vivo. The observed reduction of the relatively mild hypertensive effect of adenosine by PPADS is difficult to interpret and may either indicate that PPADS also has P1 purinoreceptor inhibitory qualities or that adenosine acts also to some extent on P2 purinoreceptors. The fact that PPADS inhibited the effects of Ap5A and at higher concentrations also partially those of ATP and adenosine, but not of ADP and AMP, further indicates that the observed decrease in blood pressure is predominantly caused by Ap5A and not by its degradation products. To further understand this pattern of antagonism of purinergic agonists by PPADS on blood pressure in vivo, complete dose-response curves would be necessary, which are, however, beyond the scope of this study. The main limitation for a conclusive interpretation is that the agonists have different affinities to the various receptor subtypes. Finally, a possible influence of PPADS via inhibition of ectonucleotidases cannot be excluded, which could decrease the breakdown of Ap5A and thus the generation of the degradation products. There are, however, no data so far demonstrating an inhibition of Ap5A breakdown via this mechanism.

In our study the Ap5A-induced hypotension was inhibited by the A1 antagonist DPCPX; an apparent inhibition of relaxation of vascular smooth muscle by DPCPX via blockade of the A1 receptor is certainly surprising. Only vasoconstriction was found to be due to A2 receptor activation. Therefore, either DPCPX blocked A2 receptors as well, although at nanomolar concentrations it is assumed to be specific for A1 receptors, or alternatively and more likely, these effects were mediated via the known negative inotropic and chronotropic effects of activation of cardiac A1 receptors by ApnA, resulting in hypotension being antagonized by DPCPX. These effects of ApnA have been described before for isolated guinea pig and human cardiac preparations (Vahlensieck et al., 1996, 1999). DMPX antagonized Ap5A-induced hypotension at about 10 times higher concentrations than DPCPX. In context with reports on an inhibition of ApnA-induced vasorelaxation by DMPX via vascular A1 receptor antagonism (van der Giet et al., 1997) this suggests an A1 antagonistic effect of DMPX on the vascular level. The pattern of antagonism of purinergic effects of Ap5A and its possible degradation products observed with DPCPX and DMPX again supports our conclusion that Ap5A most likely acts as such because only at much higher doses, these antagonists partially decreased the effects of ATP, ADP, or adenosine (DPCPX) or of ATP and adenosine (DMPX) but not of the other monoadenosine phosphates. The same above-mentioned limitations for PPADS apply for the effects of these adenosine receptor antagonists herein.

In conclusion, ApnA decrease MABP. The effect of at least Ap5A is the result of vascular P2Y1 and A2 and probably cardiac A1 purinoreceptor activation. Although the hypertensive influence of ApnA on blood pressure has been used already to lower blood pressure in humans during anesthesia (Kikuta et al., 1999), further ex vivo studies seem to be necessary to understand the physiological action of these purines and their potential as drug. Additional in vivo studies are of limited value because various complex biological systems in the organism are affected during systemic drug application and the specificity of the available antagonists is limited.

References


Lührle J and Ogilvie A (1998) Catabolism of Ap4A and Ap5A in whole blood. The dinucleotides are long-lived signal molecules in the blood ending up as intracellu-


Send reprint requests to: Prof. Dr. Eberhard Schlatter, Medizinische Poliklinik, Experimentelle Nephrologie, Westfälische Wilhelms-Universität, Domagkstrasse 3a, 48149 Münster, Germany. E-mail: eberhard.schlatter@uni-muenster.de